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(54) Title: HUMANIZED INTERLEUKIN-2 RECEPTORS ANTIBODIES

(57) Abstract

A DNA molecule, for example an expression vector, encoding at least one chain of a humanised antibody capable of binding to the human IL-2 receptor and having complementarity determining regions (CDRs) of defined amino acid sequence. The antibody itself may be used in treating a T-cell mediated disease state, for example graft versus host disease or an autoimmune disease.

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HUMANIZED INTERLEUKIN-2 RECEPTORS ANTIBODIES

The present invention relates to antibodies, more particularly humanised antibodies which bind to the interleukin-2 receptor (IL-2R).

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains.

Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al., ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

The preparation of an altered antibody in which the CDRs are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody may be derived from a human antibody. Such a humanised antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404. CAMPATH is a Registered Trade Mark of the Wellcome group of companies.

The IL-2R plays an important role in regulation of the immune response. Anti-IL-2R antibodies may therefore be immunosuppressive when administered in vivo. Anti-IL-2R antibodies may be useful as a result in the treatment of graft versus host disease, transplant rejection and autoimmune diseases. Anti-IL-2R antibodies are sometimes designated CD25 antibodies.

Non-human monoclonal antibodies have been raised which are anti-IL-2R antibodies. However, non-human monoclonal antibodies do not fix human complement particularly well and are immunogenic when injected into a human patient. Chimaeric antibodies have been proposed in WO 89/09622 which are composed of a human constant region and a mouse variable region. However, a significant immunogenicity problem remains.

WO 90/07861 relates to a method of designing a humanised immunoglobulin chain having one or more complementarity determining regions (CDRs) from a donor Ig and a framework region from a human Ig. The specification also discloses a human-like immunoglobulin specifically reactive with p55Tac protein and capable of inhibiting

binding of human IL2 to a human IL-2 receptor.

According to one aspect, the present invention provides a DNA molecule encoding at least one chain of a humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human IL-2 receptor:

light chain:	CDR1	(SEQ ID NOS: 3 and 4)
	CDR2	(SEQ ID NOS: 5 and 6)
	CDR3	(SEQ ID NOS: 7 and 8)
heavy chain:	CDR1	(SEQ ID NOS: 11 and 12)
	CDR2	(SEQ ID NOS: 13 and 14)
	CDR3	(SEQ ID NOS: 15 and 16).

According to another aspect of the present invention, there is also provided a humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human IL-2R:

light chain:	CDR1	(SEQ ID NOS: 3 and 4)
	CDR2	(SEQ ID NOS: 5 and 6)
	CDR3	(SEQ ID NOS: 7 and 8)
heavy chain:	CDR1	(SEQ ID NOS: 11 and 12)
	CDR2	(SEQ ID NOS: 13 and 14)
	CDR3	(SEQ ID NOS: 15 and 16)

The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4; or IgM, IgA, IgE or IgD. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain.

The antibody may be a chimaeric antibody of the type

described in WO 86/01533. A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain and/or heavy chain variable domain. Typically the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused to the C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The non-immunoglobulin region may be a carbohydrate region. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The light chain CDRs 1 to 3 and heavy chain CDRs 1 to 3 of SEQ ID NOS: 3 to 8 and SEQ ID NOS: 11 to 16 respectively are the CDRs of the YTH 906.9.21 antibody. YTH 906.9.21 is a rat monoclonal antibody which binds to the 55kD chain (ß-chain) of the IL-2R on activated T cells (Tighe et al., Transplantation, 45, 226-228, (1988), where the YTH 906.9.21 antibody is termed Campath-6). The specificity of a humanised antibody for the human IL-2R can be determined by inhibition of binding of ^{125}I-labelled recombinant IL-2 to PHA-activated blastocytes, by flow cytometry and/or by immunoprecipitation as described by Tighe et al.

Suitably, the CDRs of a humanised antibody are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 above. The amino acid sequences of these CDRs may be changed, however. The amino acid sequence of each CDR may be changed by up to 40% by amino acid substitutions, insertions and/or deletions, for example by up to 30%, up to 20% or up to 10%.

Each CDR may therefore include one or two amino acid substitutions, insertions and/or deletions. There may be up to three amino acid substitutions, insertions and/or deletions in light chain CDR3 or heavy chain CDR3. Up to four amino acid substitutions, insertions and/or deletions may be present in light chain CDR1. Up to six amino acid substitutions, insertions and/or deletions may be present in heavy chain CDR2. Preferably the amino acid sequence of each CDR is substantially homologous to that of each CDR of YTH 906.9.21 antibody.

The framework and the constant domains of the antibody are human framework and human constant domains. Preferably the framework of the variable region of the antibody heavy chain is substantially homologous to the corresponding framework of the human protein KOL (Schmidt et al., Hoppe-Seyler's Z. Physiol. Chem., 364, 713-747, (1983)). Homology in respect of the framework is generally 80% or more with respect to KOL, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present. For example, the seventh residue of framework 4 is suitably Thr or Leu, preferably Leu. This residue is KOL residue 109 by Kabat et al., 1987. Other candidate framework changes that may be made to restore binding include amino acid residues 27, 30, 48, 66, 67, 71, 91, 93 and 94. The amino acid numbering is according to Kabat et al.

The framework of the variable region of the antibody light chain is typically substantially homologous to the variable domain framework of the protein REI (Epp et al., Eur. J. Biochem. 45, 513-524, (1974)). Homology in respect of the framework is generally 80% or more with respect to REI, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present, for example at amino acid residue 71 according to the numbering of Kabat et al.

A humanised antibody is prepared according to the invention by a process which comprises maintaining a host transformed with a first expression vector which encodes the light chain of the humanised antibody and with a second expression vector which encodes the heavy chain of the humanised antibody under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

The first and second expression vectors may be the same vector. The invention further provides:

- a DNA sequence encoding the light chain or the heavy chain of the humanised antibody;
- an expression vector which incorporates a said DNA sequence; and
- a host transformed with a said expression vector.

Each chain of the antibody may be prepared by CDR replacement. The CDRs of a variable region of a light or heavy chain of a human antibody are replaced by sufficient of the amino acid sequence of each CDR of YTH 906.9.21 antibody such that the resulting antibody is capable of binding to the human IL-2R. The CDR-encoding regions of DNA encoding a hypervariable region of a human antibody chain are replaced by DNA encoding the desired CDRs. If appropriate, this altered DNA is linked to DNA encoding a constant domain for the antibody chain. The DNA is cloned into an expression vector. The expression vector is introduced into a compatible host cell which is cultured under such conditions that the antibody chain is expressed. Complementary antibody chains which are co-expressed in this way may then assemble to form the humanised antibody.

There are four general steps to humanise a monoclonal antibody. These are:

(1) determining the nucleotide and pr dicted amino acid sequence of the starting antibody light and heavy variable

domains;

(2) designing the humanised antibody, i.e. deciding which antibody framework region to use during the humanising process;

(3) the actual humanising methodologies/techniques; and

(4) the transfection and expression of the humanised antibody.

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody only the amino acid sequence of antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs:

(1) via a conventional cDNA library, or

(2) via the polymerase chain reaction (PCR).

Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains. In the present instance, the nucleotide sequence and predicted amino acid sequence of the light and heavy chains of the rodent YTH 906.9.21 antibody are shown in SEQ ID NOS: 1 and 2 and SEQ ID NOS: 9 and 10.

Step 2: Designing the humanised antibody

There are several factors to consider in deciding

which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.

2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is

performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable domain which is most homologous is chosen as the framework for humanisation.

Step 3: The actual humanising methodologies/techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A-0239400. A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesized that can be used to mutagenize the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro

mutagenesis is well known.

Alternatively, humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO 92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of WO 92/07075 can be performed using a template comprising two human framework regions, AB and CD, and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5′ ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5′ ends to each other under conditions which allow a PCR to be performed. Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

Step 4: <u>The transfection and expression of the reshaped antibody</u>

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs

required for the humanised antibody of the invention;

(b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both prepared vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the human antibody chain. The humanised antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. For single antibody chains, it is envisaged that E. coli - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to

standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, a humanised antibody may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The invention has been described herein specifically with reference to a humanised antibody having one or more CDRs all or part of which are derived from the rat monoclonal antibody YTH 906.9.21. However, the procedures and techniques described herein are equally applicable to the production of humanised antibodies having CDRs derived from antibodies other than YTH 906.9.21 and capable of binding to the human IL-2 receptor.

Accordingly, according to a further aspect, the present invention provides a humanised (CDR-grafted) anti-IL-2R antibody.

The IL-2R specific antibodies typically find use in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as bearing the IL-2R, then the humanised antibodies capable of binding the IL-2R are suitable. For example, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as

Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The human-like antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984).

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

An antibody of the present invention may form part of an immunotoxin. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle", provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a prot in and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various

immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet", Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). See, generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25, 335-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985).

The delivery component of the immunotoxin is a humanised antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgA, IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The invention further provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a humanised antibody according to the invention. The composition may comprise an immunotoxin according to the invention. The humanised antibody, immunotoxin and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously,

intramuscularly or intravenously.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with

conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose". Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of the invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human-like antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose". In this

use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

Human-like antibodies of the present invention can further find a wide variety of utilities in vitro. By way of example, the antibodies can be utilized for T-cell typing, for isolating specific IL-2R bearing cells or fragments of the receptor, for vaccine preparation, or the like.

For diagnostic purposes, the antibodies may either be labelled or unlabelled. Unlabelled antibodies can be used in combination with other labelled antibodies (second antibodies) that are reactive with the humanised antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labelled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, a humanised antibody of the present

invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above. Generally the kit will also contain a set of instructions for use.

The following Example illustrates the invention.

EXAMPLE

Cloning and sequencing of the YTH 906.9.21 antibody light chain

PolyA+ mRNA was made by the method of Auffray and Rougeon, Eur. J. Biochem, 107, 303-314, (1980) and cDNA by that of Gubler and Hoffman, Gene 25, 263-269 (1983) except using an oligonucleotide shown in SEQ ID NO: 35 designed to selectively prime on rat constant regions (DA allotype) in place of oligodT primer. The cDNA was C-tailed with terminal transferase and ligated into a G-tailed vector (the vector was derived from pUC8 and first digested with

SmaI). Colonies were screened by restriction digestion and any inserts of suitable size (>300 bp) subcloned into the vector M13mp19 (Yanisch-Perron et al., Gene, 33, 103-119) and sequenced using the dideoxy method (Sanger et al., Proc. Natl. Acad. Sci. USA, 74, 5463-5467 (1977)). The sequence of the variable region is shown in SEQ ID NOS: 1 and 2.

Cloning and sequencing of the YTH 906.9.21 antibody heavy chain

Total RNA was isolated from 2.5 x 10^7 cells expressing YTH 906.9.21 antibody following the method of Chomczynski and Sacchi (Anal. Biochem., 162, 156-159, (1987)), using 1ml of extraction solution per 1 x 10^7 cells. The resulting RNA pellet was redissolved in 50μl diethyl pyrocarbonate (DEPC)-treated distilled water, and spectrophotometrically determined to be at a concentration of 4μg/μl. Dynabeads Oligo $(dT)_{25}$ (Dynal) was used to extract mRNA from 75μg total RNA employing the manufacturer's protocol.

cDNA was synthesized from the isolated mRNA and cloned into the plasmid pSPORT-1 using the SUPERSCRIPT Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL) following the method recommended by the manufacturer. Escherichia coli MAX EFFICIENCY DH5α Competent Cells (BRL) were transformed with the resulting cDNA/pSPORT-1 ligation. Approximately 5000 colonies were lifted onto Hybond-N nylon filters (Amersham) and lysed, denatured and fixed following the method of Buluwela et al., (Nucleic Acids Res. 17, 452, (1989)). The filters were treated with proteinase K (50μg/ml) in 0.2 x SSC, 0.1% SDS at 55°C for 30 min, and then excess debris removed with a tissue.

A rat heavy chain clone was labelled with digoxigenin-11-dUTP using the Nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim) and employed to

screen the filters for YTH 906.9.21 heavy chain following the manufacturer's protocol. Approximately 250 potential positive colonies were detected, and 12 selected for further analysis. Plasmid DNA was prepared using the method of Del Sal et al., (Nucleic Acids Res., 16, 9878, (1988)) and 8 of the 12 contained inserts of the expected size for rat immunoglobulin heavy chain cDNA. A clone, pC6H3, was selected, and sequenced in both directions by plasmid priming following the dideoxy chain termination method (Sanger et al., (1977) supra), according to the sequenase kit (USB) protocol. The sequence of the variable region is shown in SEQ ID NOS: 9 and 10.

Designing the chimaeric antibody

Using the selection procedure described in Step 2 above, the human variable domain frameworks of the KOL heavy chain and REI light chain (Kabat et al., 1987) were chosen for the humanisation process.

Construction of the humanised heavy and light chain genes

The humanised heavy and light chains were constructed following the method of Lewis and Crowe (Gene, 101, 297-302, (1991)).

(i) Light Chain

Light chain oligonucleotide primers:

A_L: SEQ ID NO: 17:

B_L: SEQ ID NO: 18:

C_L: SEQ ID NO: 19:

D_L: SEQ ID No: 20:

E_L: SEQ ID NO: 21:

F_L: SEQ ID NO: 22:

G_L: SEQ ID NO: 23:

H_L: SEQ ID NO: 24:

PCR reactions (Saiki et al., Science, 239, 487-491, (1988)) were performed in a programmable heating block (Hybaid) using 20 rounds of temperature cycling (94°C for 1 min, 50°C for 2 min, and 72°C for 3 min) followed by a final 10 min step at 72°C. 1μg of each primer, a specified amount of template, and 2.5 units of Taq polymerase (Perkin Elmer Cetus) were used in a final volume of 100μl with the reaction buffer as recommended by the manufacturer.

The initial template for the PCR was CAMPATH-1H light chain (humanised CAMPATH-1 on REI framework; Page and Sydenham, Bio/Technology, 9, 64, (1991)). Four primary PCR reactions were initially carried out, with 10ng of template per reaction, using the primer pairs A_L with B_L, C_L with D_L, E_L with F_L, and G_L with H_L respectively. The products of these PCR reactions, fragments AB_L, CD_L, EF_L and GH_L respectively, were purified using Prep-A-Gene (Bio-Rad) following the protocol recommended by the manufacturer. Fragments AB_L with CD_L, and EF_L with GH_L were combined using a quarter of each purified product, and subjected to recombinant PCR reactions with primers A_L plus D_L, and E_L plus H_L respectively. The products of these reactions, fragments AD_L and EH_L, were purified as above, and a quarter of each combined in a recombinant PCR reaction using primers A_L and H_L. The final humanised light chain recombinant PCR product, AH_L, was cloned into the HindIII site of pUC-18 (BRL) following the method of Crowe et al., Nucleic Acids Res., 19, 184 (1991), utilising the HindIII sites in primers A_L and H_L. Plasmid isolates were sequenced by the dideoxy chain termination method, and clones of the correct sequence chosen.

(ii) Heavy Chain

Heavy chain oligonucleotide primers:

A_H: SEQ ID NO: 25:

B_H: SEQ ID NO: 26:

C_H: SEQ ID NO: 27:

D_H: SEQ ID NO: 28:
E_H: SEQ ID NO: 29:
F_H: SEQ ID NO: 30:
G_H: SEQ ID NO: 31:
H_H: SEQ ID NO: 32:

The initial template for the PCR was humanised anti-CD4 heavy chain (on KOL framework; WO 92/05274) subsequently converted from genomic to cDNA context. The rodent CDR's were grafted on to the template following the recombinant PCR method as described above, but using oligonucleotide primers A_H to H_H. Oligonucleotides A_H and H_H were designed with HindIII and EcoRI sites respectively to enable initial cloning of the humanised variable region, and a SpeI site was introduced into the KOL framework 4 (FR4) region of oligonucleotide G_H to facilitate subsequent cloning of the variable region with a suitable constant region of choice. The SpeI site altered the threonine residue at position 109 (numbering according to Kabat et al., 1987) of the humanised anti-CD4 heavy chain template (proline in KOL) to a leucine residue (four out of the six human heavy J-minigenes possess a leucine at this position; Kabat et al., 1987).

The humanised heavy chain variable region recombinant PCR product was cloned into HindIII/EcoRI-cut pUC-18 (BRL), and plasmid isolates of the correct sequence were chosen. The FR4 and $\gamma 1$ constant regions of the humanised anti-CD4 heavy chain were PCR cloned into pUC-18 (BRL) using oligonucleotide primers X_H (SEQ ID NO: 33) and Y_H (SEQ ID NO: 34). Primer X_H contains SpeI and HindIII sites, and Y_H an EcoRI site. The HindIII and EcoRI sites were used to clone the PCR product into pUC-18, and plasmid isolates of the correct sequence were selected. The complete heavy chain was subsequently reconstituted from the humanised variable region and $\gamma 1$ constant region clones using the engineered FR4 SpeI site.

SEQUENCE LISTING

(1) INFORMATION FOR SEQ ID NO : 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH	: 321 base pairs
(B) TYPE	: nucleic acid
(C) STRANDEDNESS	: double
(D) TOPOLOGY	: linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM	: Rattus rattus

(ix) FEATURE:

(A) NAME/KEY	: CDS
(B) LOCATION	: 1..321
(D) OTHER INFORMATION	: /label = variable region of YTH 906.9.21 antibody

(ix) FEATURE:

(A) NAME/KEY	: misc_feature
(B) LOCATION	: 70..102
(D) OTHER INFORMATION	: /label = CDR 1

(ix) FEATURE:

(A) NAME/KEY	: Misc_feature
(B) LOCATION	: 148..168
(D) OTHER INFORMATION	: /label = CDR 2

(ix) FEATURE:

(A) NAME/KEY : Misc_feature
(B) LOCATION : 265..291
(D) OTHER INFORMATION : /label = CDR 3

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 1:

```
TAC ATC CAG ATG ACA CAG TCT CCT CCC TCT CTG TCT GCA TCT CTG GGA     48
Tyr Ile Gln Met Thr Gln Ser Pro Pro Ser Leu Ser Ala Ser Leu Gly
 1               5                  10                  15

GAC AAA GTC ACC ATC ACT TGC CAG GCA AGT CAA AAC ATT AAC AAG TAT     96
Asp Lys Val Thr Ile Thr Cys Gln Ala Ser Gln Asn Ile Asn Lys Tyr
            20                  25                  30

ATA GCT TGG TTT CAG CAA AAG CCT GGA AAA ACT CCT AGG CAG CTC ATA    144
Ile Ala Trp Phe Gln Gln Lys Pro Gly Lys Thr Pro Arg Gln Leu Ile
        35                  40                  45

CAT TAC ACA TCT ACA CTA GTG TCA GGC ATC CCA TCG AGG TTC AGT GGC    192
His Tyr Thr Ser Thr Leu Val Ser Gly Ile Pro Ser Arg Phe Ser Gly
    50                  55                  60

AGT GGA TCT GGG AAA GAT TAT TCA TTC AGC ATC AGC AAC GTG GAG TCT    240
Ser Gly Ser Gly Lys Asp Tyr Ser Phe Ser Ile Ser Asn Val Glu Ser
65                  70                  75                  80

GAA GAT ATT GCA AAT TAT TAC TGT CTA CAG TAC GAT GAA ATT CCG AAC    288
Glu Asp Ile Ala Asn Tyr Tyr Cys Leu Gln Tyr Asp Glu Ile Pro Asn
                85                  90                  95

ACG TTT GGA CCT GGG ACC AAG CTG GAG CTG AAA                       321
Thr Phe Gly Pro Gly Thr Lys Leu Glu Leu Lys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO : 2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 107 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 2:

Tyr Ile Gln Met Thr Gln Ser Pro Pro Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Asp Lys Val Thr Ile Thr Cys Gln Ala Ser Gln Asn Ile Asn Lys Tyr
20 25 30

Ile Ala Trp Phe Gln Gln Lys Pro Gly Lys Thr Pro Arg Gln Leu Ile
35 40 45

His Tyr Thr Ser Thr Leu Val Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Lys Asp Tyr Ser Phe Ser Ile Ser Asn Val Glu Ser
65 70 75 80

Glu Asp Ile Ala Asn Tyr Tyr Cys Leu Gln Tyr Asp Glu Ile Pro Asn
85 90 95

Thr Phe Gly Pro Gly Thr Lys Leu Glu Leu Lys
100 105

(3) INFORMATION FOR SEQ ID NO : 3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..33

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..33
(D) OTHER INFORMATION : /label = CDR 1 light chain

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 3:

```
CAG GCA AGT CAA AAC ATT AAC AAG TAT ATA GCT                    33
Gln Ala Ser Gln Asn Ile Asn Lys Tyr Ile Ala
 1               5                  10
```

(4) INFORMATION FOR SEQ ID NO : 4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 11 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 4:

```
Gln Ala Ser Gln Asn Ile Asn Lys Tyr Ile Ala
 1               5                  10
```

(5) INFORMATION FOR SEQ ID NO : 5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 21 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..21
(D) OTHER INFORMATION : /label = CDR 2 light chain

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 5:

```
TAC ACA TCT ACA CTA GTG TCA                                    21
Tyr Thr Ser Thr Leu Val Ser
 1               5
```

(6) INFORMATION FOR SEQ ID NO : 6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 7 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 6:

```
Tyr Thr Ser Thr Leu Val Ser
 1               5
```

(7) INFORMATION FOR SEQ ID NO : 7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 27 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..27
(D) OTHER INFORMATION : /label = CDR 3 light chain

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 7:

```
CTA CAG TAC GAT GAA ATT CCG AAC ACG                                    27
Leu Gln Tyr Asp Glu Ile Pro Asn Thr
 1               5
```

(8) INFORMATION FOR SEQ ID NO : 8

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 9 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 8:

Leu Gln Tyr Asp Glu Ile Pro Asn Thr
1 5

(9) INFORMATION FOR SEQ ID NO : 9

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 351 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..351
(D) OTHER INFORMATION : /label = variable region of YTH 906.9.21 antibody

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 91..105
(D) OTHER INFORMATION : /label = CDR 1

(ix) FEATURE:

(A) NAME/KEY : Misc_feature
(B) LOCATION : 148..198

(D) OTHER INFORMATION : /label = CDR 2

(ix) FEATURE:

(A) NAME/KEY : Misc_feature
(B) LOCATION : 295..318
(D) OTHER INFORMATION : /label = CDR 3

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 9:

```
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTA GTG CAG CCT GGA AGG     48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
  1               5                  10                  15

TCC CTG AAA CTC TCC TGT GCA GCC TCA GGA TTC ACT TTC AGT GAC TAT     96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
             20                  25                  30

TAC ATG GCC TGG GTC CGC CAG GCT CCA ACG AAG GGT CTG GAG TGG GTC    144
Tyr Met Ala Trp Val Arg Gln Ala Pro Thr Lys Gly Leu Glu Trp Val
         35                  40                  45

GCA TCC ATT AGT TAT GAT GGT GGT GCC ACT TAC TAT CGA GAC TCC GTG    192
Ala Ser Ile Ser Tyr Asp Gly Gly Ala Thr Tyr Tyr Arg Asp Ser Val
     50                  55                  60

AAG GGC CGA TTT ACT ATC TCC AGA GAT AAT GCA AAA AGC AGC CTA TAC    240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr
 65                  70                  75                  80

CTG CAG GTG GAC GGT CTG AGG TCT GAG GAC ACG GCC ACT TAT TAC TGT    288
Leu Gln Val Asp Gly Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
                 85                  90                  95

ACA ACG GGT AGC AGC TTC ACC GGG GGT TAC TGG GGC CAA GGA GTC ATG    336
Thr Thr Gly Ser Ser Phe Thr Gly Gly Tyr Trp Gly Gln Gly Val Met
            100                 105                 110

GTC ACA GTC TCC TCA                                               351
Val Thr Val Ser Ser
            115
```

(10) INFORMATION FOR SEQ ID NO : 10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 117 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 10:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
 1               5                  10                  15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30

Tyr Met Ala Trp Val Arg Gln Ala Pro Thr Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Ser Ile Ser Tyr Asp Gly Gly Ala Thr Tyr Tyr Arg Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr
65                  70                  75                  80

Leu Gln Val Asp Gly Leu Arg Ser Glu Asp Thr Ala Thr Tyr Tyr Cys
                85                  90                  95

Thr Thr Gly Ser Ser Phe Thr Gly Gly Tyr Trp Gly Gln Gly Val Met
            100                 105                 110

Val Thr Val Ser Ser
        115
```

(11) INFORMATION FOR SEQ ID NO : 11

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 15 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..15
(D) OTHER INFORMATION : /label = CDR 1 heavy chain

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 11:

```
GAC TAT TAC ATG GCC                                              15
Asp Tyr Tyr Met Ala
 1               5
```

(12) INFORMATION FOR SEQ ID NO : 12

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 5 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 12:

```
Asp Tyr Tyr Met Ala
 1               5
```

(13) INFORMATION FOR SEQ ID NO : 13

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 51 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..51
(D) OTHER INFORMATION : /label = CDR 2 heavy chain

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 13:

```
TCC ATT AGT TAT GAT GGT GGT GCC ACT TAC TAT CGA GAC TCC GTG AAG    48
Ser Ile Ser Tyr Asp Gly Gly Ala Thr Tyr Tyr Arg Asp Ser Val Lys
 1               5                  10                  15

GGC                                                              51
Gly
```

(14) INFORMATION FOR SEQ ID NO : 14

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 17 amino acids
(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 14:

Ser Ile Ser Tyr Asp Gly Gly Ala Thr Tyr Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

(15) INFORMATION FOR SEQ ID NO : 15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 24 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..21
(D) OTHER INFORMATION : /label = CDR 3
heavy chain

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 15:

GGT AGC AGC TTC ACC GGG GGT TAC 24
Gly Ser Ser Phe Thr Gly Gly Tyr
1 5

(16) INFORMATION FOR SEQ ID NO : 16

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 7 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 16:

Gly Ser Ser Phe Thr Gly Gly Tyr
1 5

(17) INFORMATION FOR SEQ ID NO : 17

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 17:

GATCAAGCTT CTCTACAGTT ACTGAGCACA 30

(18) INFORMATION FOR SEQ ID NO : 18

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 18:

AGCTATATAC TTGTTAATGT TTTGACTTGC CTGACAGGTG ATGGTCAC 48

(19) INFORMATION FOR SEQ ID NO : 19

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 19:

CAGGCAAGTC AAAACATTAA CAAGTATATA GCTTGGTACC AGCAGAAG 48

(20) INFORMATION FOR SEQ ID NO : 20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 20:

TGACACTAGT GTAGATGTGT AGTAGATCAG CAGCTT 36

(21) INFORMATION FOR SEQ ID NO : 21

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 21:

TACACATCTA CACTAGTGTC AGGTGTGCCA AGCAGA 36

(22) INFORMATION FOR SEQ ID NO : 22

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 42 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 22:

CGTGTTCGGA ATTTCATCGT ATTGTAGGCA GTAGTAGGTG GC 42

(23) INFORMATION FOR SEQ ID NO : 23

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 42 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 23:

CTACAATACG ATGAAATTCC GAACACGTTC GGCCAAGGGA CC 42

(24) INFORMATION FOR SEQ ID NO : 24

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 24:

GATCAAGCTT CTAACACTCT CCCCTGTTGA 30

(25) INFORMATION FOR SEQ ID NO : 25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 31 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 25:

TGGGATCGAT CAAGCTTTAC AGTTACTGAG C 31

(26) INFORMATION FOR SEQ ID NO : 26

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 26:

GGCCATGTAA TAGTCACTGA AGATGAATCC 30

(27) INFORMATION FOR SEQ ID NO : 27

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 27:

GACTATTACA TGGCCTGGGT CCGCCAGGCT 30

(28) INFORMATION FOR SEQ ID NO : 28

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 28:

ATAGTAAGTG GCACCACCAT CATAACTAAT GGATGCGACC CACTCCAG 48

(29) INFORMATION FOR SEQ ID NO : 29

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 29:

GGTGCCACTT ACTATCGAGA CTCCGTGAAG GGCCGATTCA CTATCTCC 48

(30) INFORMATION FOR SEQ ID NO : 30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 39 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 30:

GTAACCCCCG GTGAAGCTGC TACCTCTTGC ACAGAAATA 39

(31) INFORMATION FOR SEQ ID NO : 31

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 54 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 31:

GGTAGCAGCT TCACCGGGGG TTACTGGGGC CAAGGGTCAC TAGTCACAGT CTCC 54

(32) INFORMATION FOR SEQ ID NO : 32

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 32:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG 36

(33) INFORMATION FOR SEQ ID NO : 33

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 33:

GCTGCTCCTT TTAAGCTTTG GGGTCAAGGC TCACTAGTCA CAGTCTCC 48

(34) INFORMATION FOR SEQ ID NO : 34

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 33 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 34:

AAGCTTCCGT CGAATTCATT TACCCGGAGA CAG 33

(35) INFORMATION FOR SEQ ID NO : 35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 17 bases

(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 35:

GTGGCACCTC CAGATGT 17

CLAIMS:

1. A DNA molecule encoding at least one chain of a humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human IL-2 receptor:

light chain: CDR1 (SEQ ID NOS: 3 and 4)
CDR2 (SEQ ID NOS: 5 and 6)
CDR3 (SEQ ID NOS: 7 and 8)

heavy chain: CDR1 (SEQ ID NOS: 11 and 12)
CDR2 (SEQ ID NOS: 13 and 14)
CDR3 (SEQ ID NOS: 15 and 16).

2. A DNA molecule as claimed in Claim 1, encoding at least the light chain of an antibody in which the variable domain framework is substantially homologous to the variable domain framework of the protein REI.

3. A DNA molecule as claimed in Claim 1 or 2, encoding at least the heavy chain of an antibody in which the variable domain framework is substantially homologous to the variable domain framework of the protein KOL.

4. A DNA molecule as claimed in any one of Claims 1 to 3, encoding the light chain of an antibody in which the CDRs are the light chain CDRs 1 to 3 specified in Claim 1 and/or encoding the heavy chain of an antibody in which the CDRs are the heavy chain CDRs 1 to 3 specified in Claim 1.

5. A DNA molecule as claimed in any of Claims 1 to 4 which is an expression vector in which the DNA encoding the said at least one chain of an antibody is in a form capable of expression in a suitable host.

6. A DNA molecule as claimed in Claim 5 which includes DNA encoding both the heavy and light chains of the antibody.

7. A host transformed with an expression vector as claimed in Claim 6.

8. A DNA molecule as claimed in Claim 5 which includes DNA encoding either the heavy chain or the light chain of the antibody.

9. A host transformed with two expression vectors as claimed in Claim 8, one encoding the heavy chain of the antibody and the other encoding the light chain of the antibody.

10. A humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human IL-2R:

light chain:	CDR1 (SEQ ID NOS: 3 and 4)
	CDR2 (SEQ ID NOS: 5 and 6)
	CDR3 (SEQ ID NOS: 7 and 8)
heavy chain:	CDR1 (SEQ ID NOS: 11 and 12)
	CDR2 (SEQ ID NOS: 13 and 14)
	CDR3 (SEQ ID NOS: 15 and 16).

11. An antibody as claimed in Claim 10, in which the variable domain framework of the light chain is substantially homologous to the variable domain framework of the protein REI.

12. An antibody as claimed in Claim 10 or 11, in which the variable domain framework of the heavy chain is substantially homologous to the variable domain framework of the protein KOL.

13. An antibody as claimed in any one of Claims 10 to 12, in which the CDRs are the light chain CDRs 1 to 3 specified in Claim 10 and the heavy chain CDRs 1 to 3 specified in Claim 10.

14. A process for the preparation of a humanised antibody as defined in any of Claims 10 to 13, which process comprises:

(i) maintaining a host transformed with a first expression vector which encodes the light chain of the humanised antibody and with a second expression vector which encodes the heavy chain of the humanised antibody; or

(ii) maintaining a host transformed with an expression vector which encodes both the light chain and the heavy chain of the humanised antibody;

under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

15. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a humanised antibody as defined in any of Claims 10 to 13.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01258

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/13; C12P21/08; A61K39/395; C12N5/10
C07K15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	WO,A,9 007 861 (PROTEIN DESIGN LABS INC, US) 26 July 1990 cited in the application see page 1 - page 6; claims 1-22	1-15
Y	TRANSPLANTATION PROCEEDINGS vol. 23, no. 1, February 1991, pages 1390 - 1392 Friend PJ;Waldmann H;Cobbold S;Tighe H;Rebello P;Wight D;Gore S;Pollard S;Lim S;Johnston P 'The anti-IL-2 receptor monoclonal antibody YTH-906 in liver transplantation' See page 1390, first column	1-15

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° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
08 OCTOBER 1992	26 NOV 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	NATURE. vol. 332, 24 March 1988, LONDON GB pages 323 - 327 Riechmann L;Clark M;Waldmann H;Winter G 'Reshaping human antibodies for therapy.' see the whole document	1-15
Y	TRANSPLANTATION vol. 45, no. 1, January 1988, pages 226 - 228 Tighe H;Friend PJ;Collier SJ;Decurtins M;Lim S;Cobbold SP;Thiru S;Calne RY;Waldmann H 'Delayed allograft rejection in primates treated with anti-IL-2 receptor monoclonal antibody Campath-6.' cited in the application see the whole document	1-15
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, December 1989, WASHINGTON US pages 10029 - 10033 Queen C;Schneider WP;Selick HE;Payne PW;Landolfi NF;Duncan JF;Avdalovic NM;Levitt M;Junghans RP;Waldmann TA 'A humanized antibody that binds to the interleukin 2 receptor.' see the whole document	1-15

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201258 SA 62138

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 08/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9007861	26-07-90	AU-A- 5153290	13-08-90
		CA-A- 2006865	28-06-90
		EP-A- 0451216	16-10-91